Detection of sparse life in extreme environments

PI Meeting Boulder Co
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Goal of project

Develop technology for search for life on Mars

Fluorescent reagents for detection of life

- 4 probes: DNA, protein, membrane, carbohydrate
- Each diff color, fluorescent only when bound

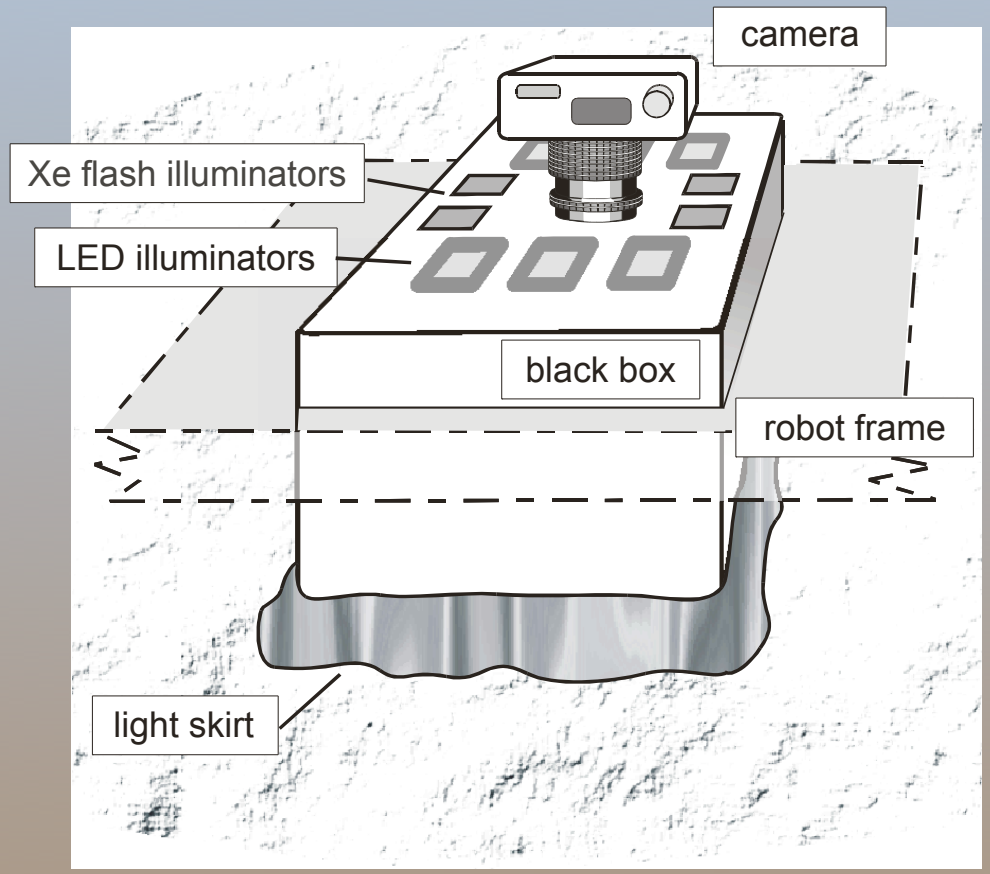
Fluorescence imaging microscope system on rover

Atacama Desert (Chile) as model

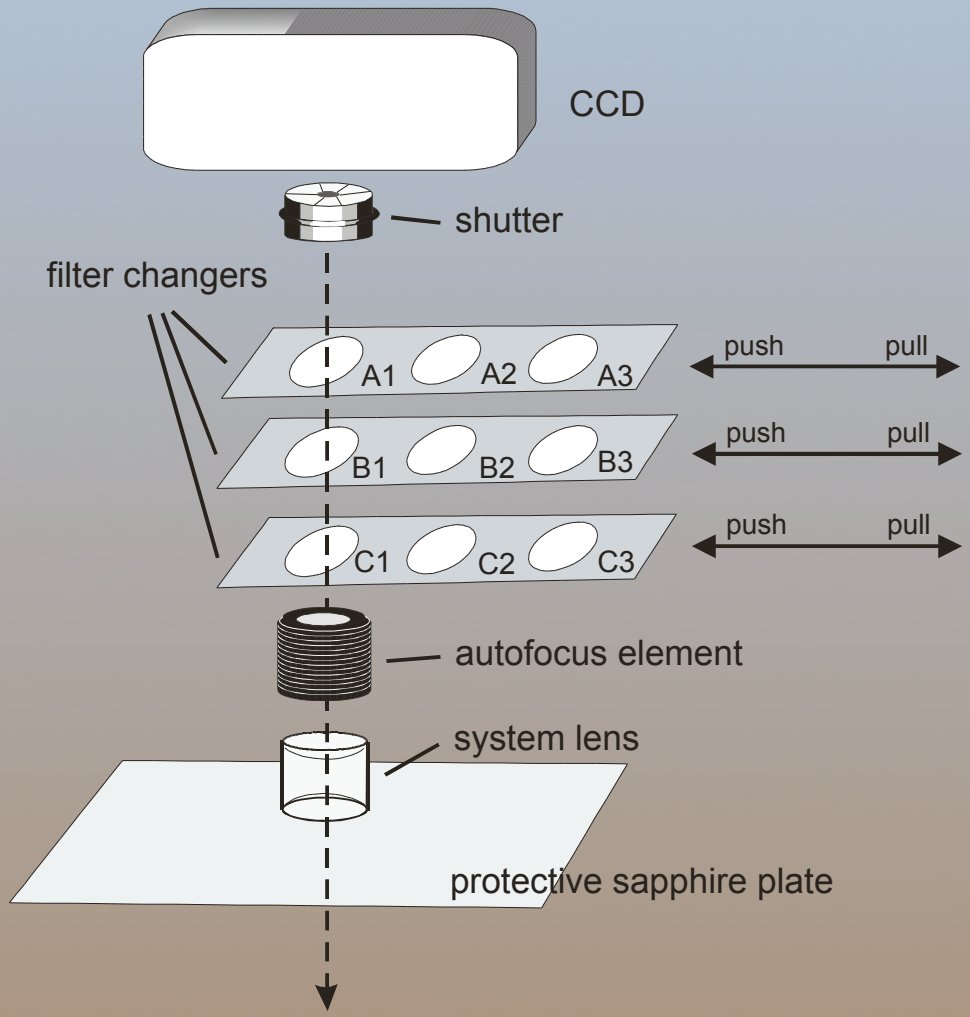
First field trip in April this year

Models Mars expedition but includes ground truth

Proposed fluorescence imaging system

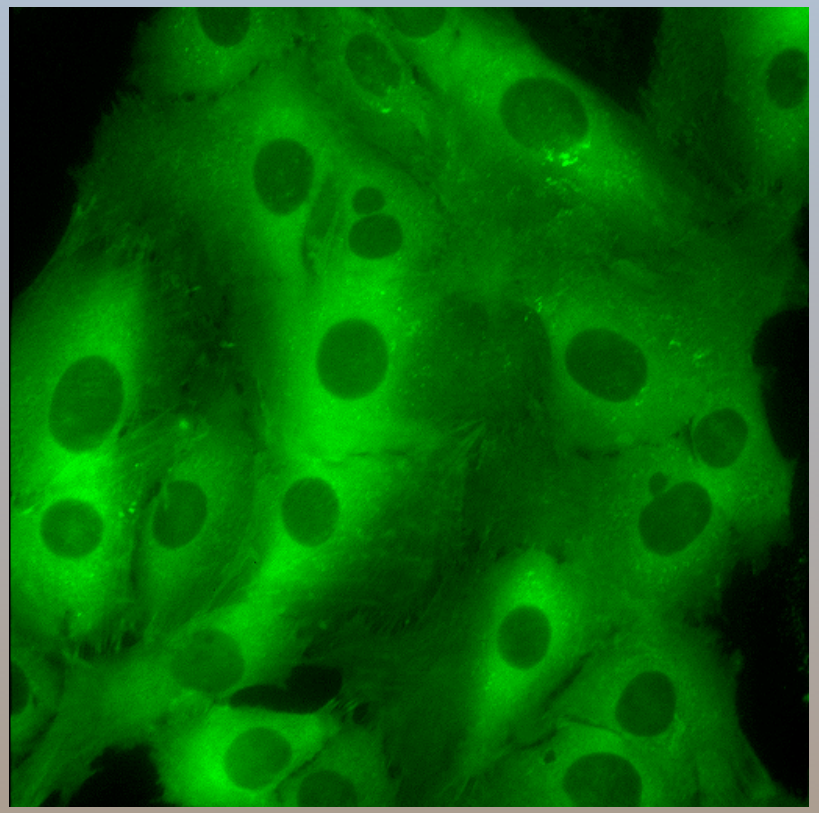


Proposed fluorescence imaging system - 2

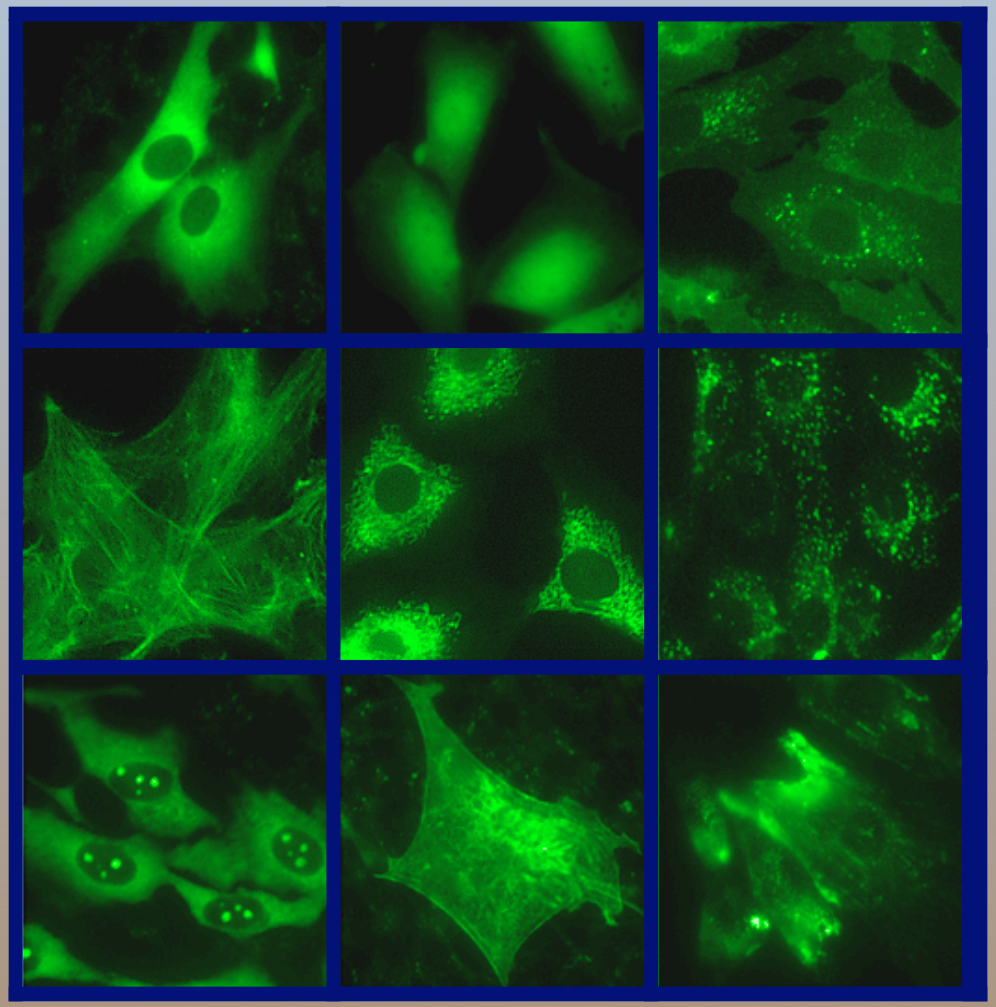


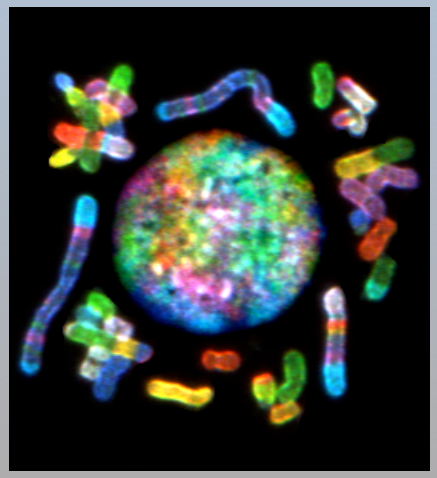
Fluorescent probe classes

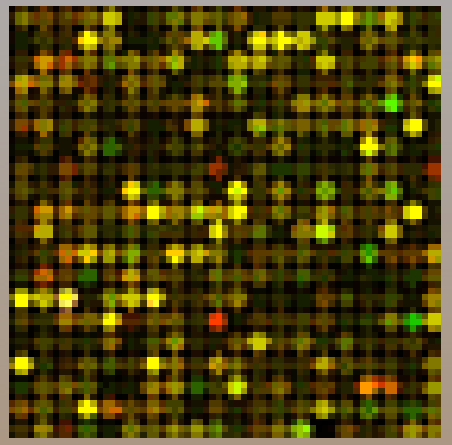
- **Covalent labels**
- **Non-covalent labels**
- **Live cell indicators**
  - Ion concentrations
  - Gene expression
  - Membrane potential
  - Cell tracking
  - Protein conformation/ interaction
  - Kinase-phosphatase indicators
  - Cell and mitochondrial energetics
  - Enzyme activity indicators
  - Protein concentration



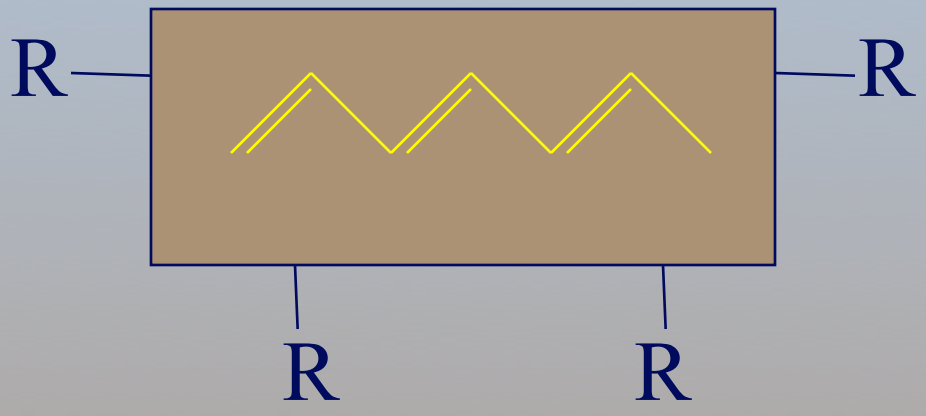
Power of fluorescent probes:







Engineering fluorescent probes

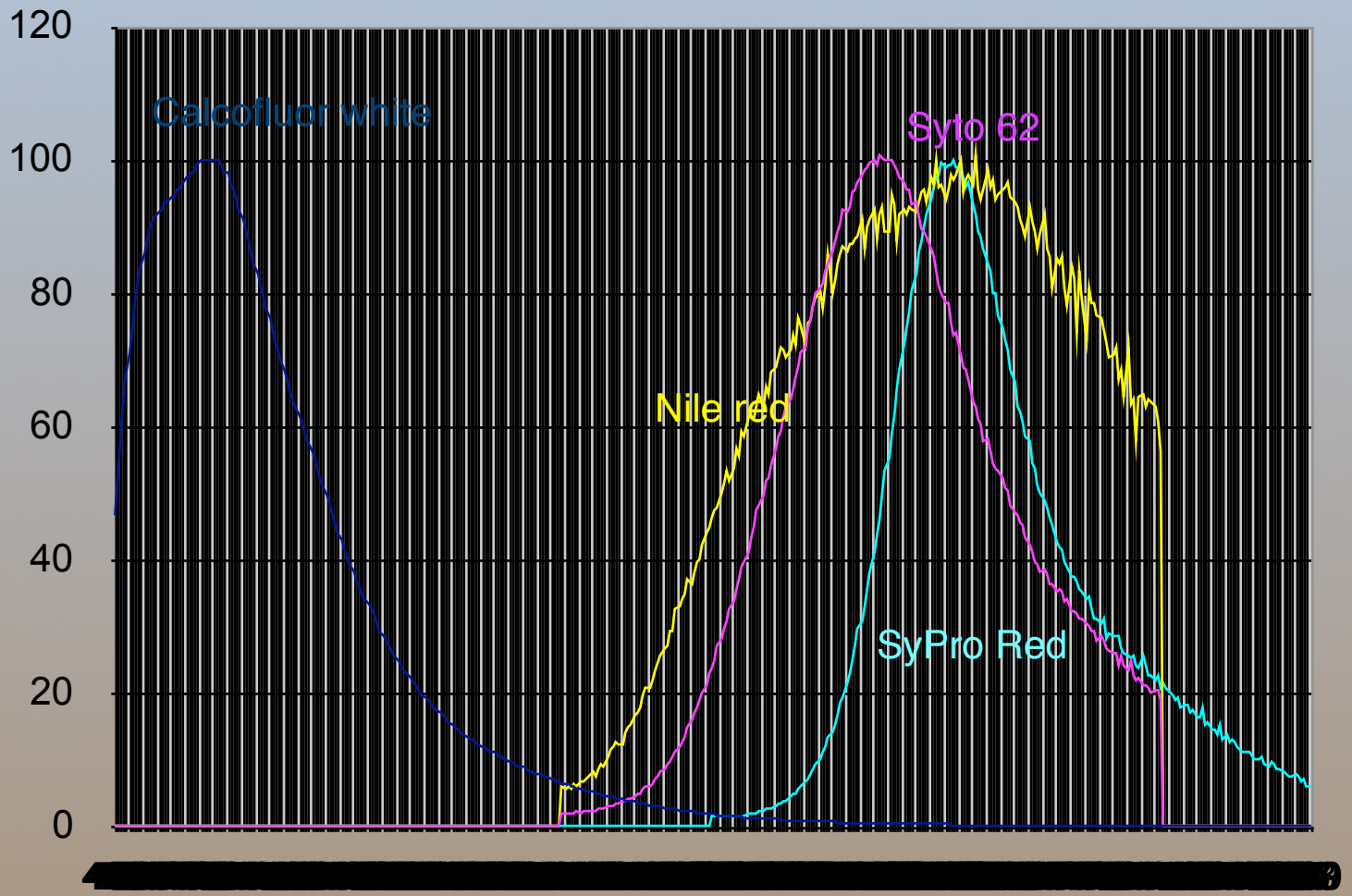


- Alkane group
- Sulfonate group
- Reactive group

Fluorescent probes

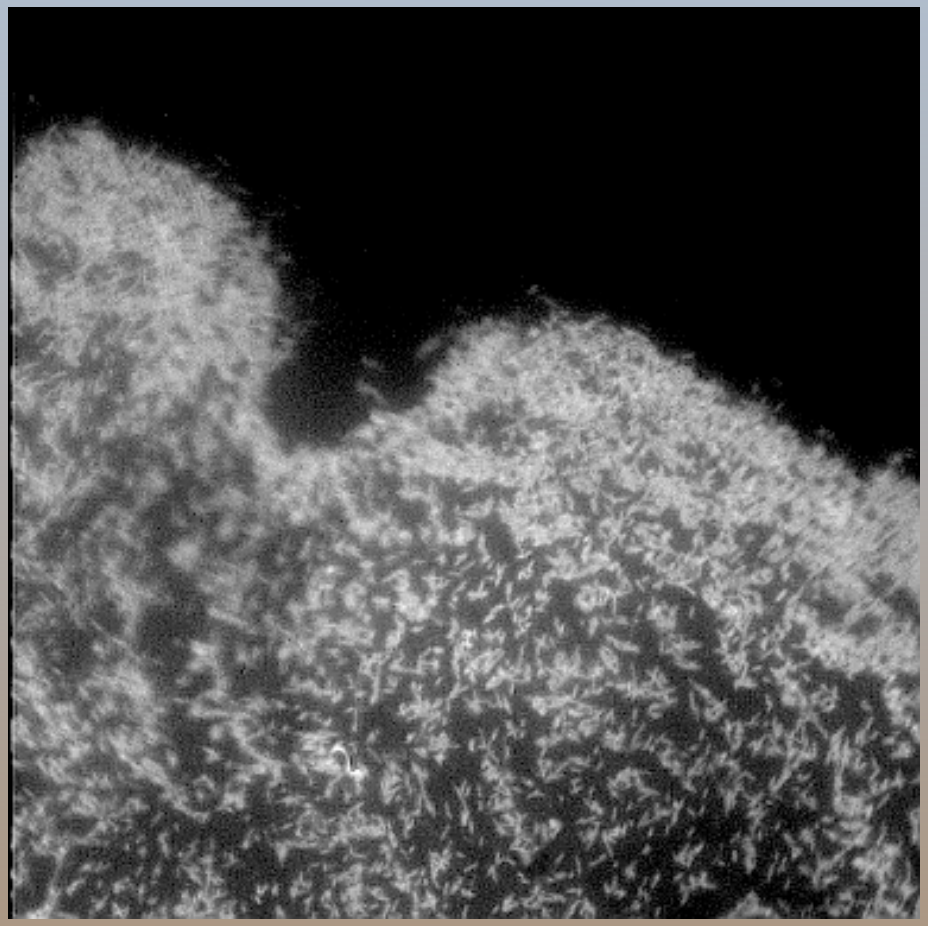
Dye	Absorption max (nm)	Emission max (nm)		Enhancement on binding	
DNA probes					
SYTO 9	470	530		++++	
SYTO BC	480	500		++++	
DRAQ56	506	700			
Protein probes					
Fluorescamine	380	464		++++	
OPA/2ME	334	455		+++	
NDA/CN	419	493		+++	
CBQCA/CN		465	560		+++
SYPRO Orange	**470**	**570**		+++	
SYPRO Red		550	630		++++
Lipid probes					
DPH	353	449		+++	
Nile Red	559	637		+++	
DiI(7)	750	780		+	
Carbohydrate probe					
Calcofluor White	360	480		+++	

Fluorescent probes on E. coli



Detection of single bacteria

Figure 1: E. coli at edge of biofilm visualized using SYPRO Red dye.



Chlorophyll fluorescence also a probe

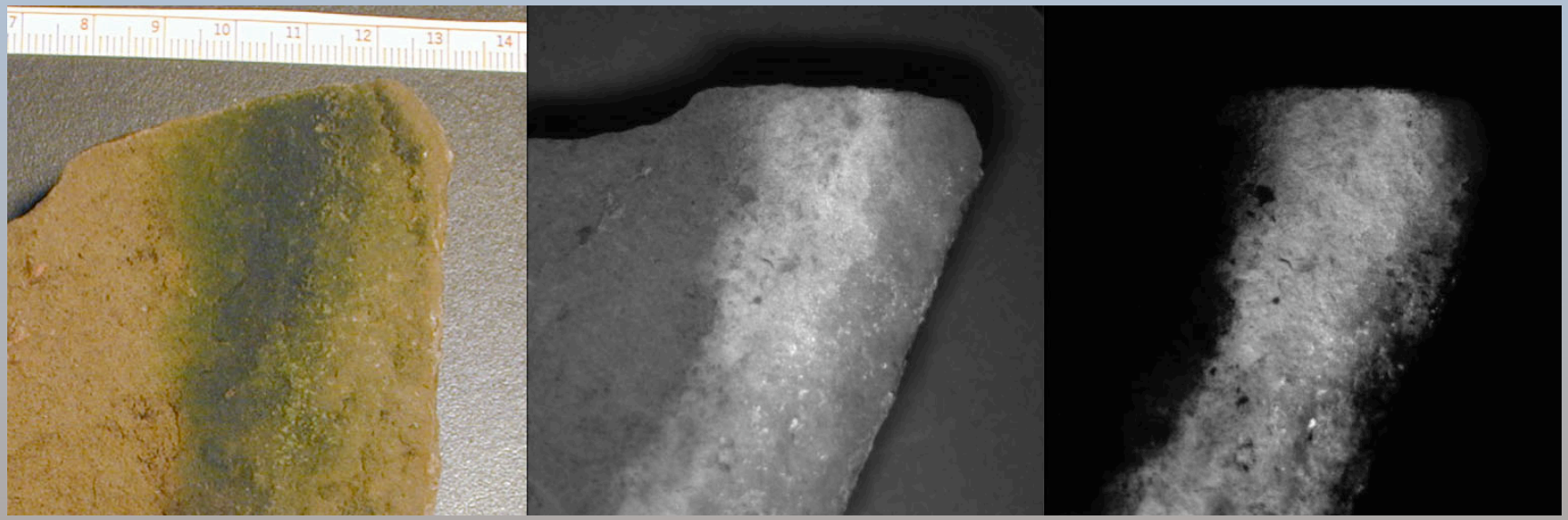
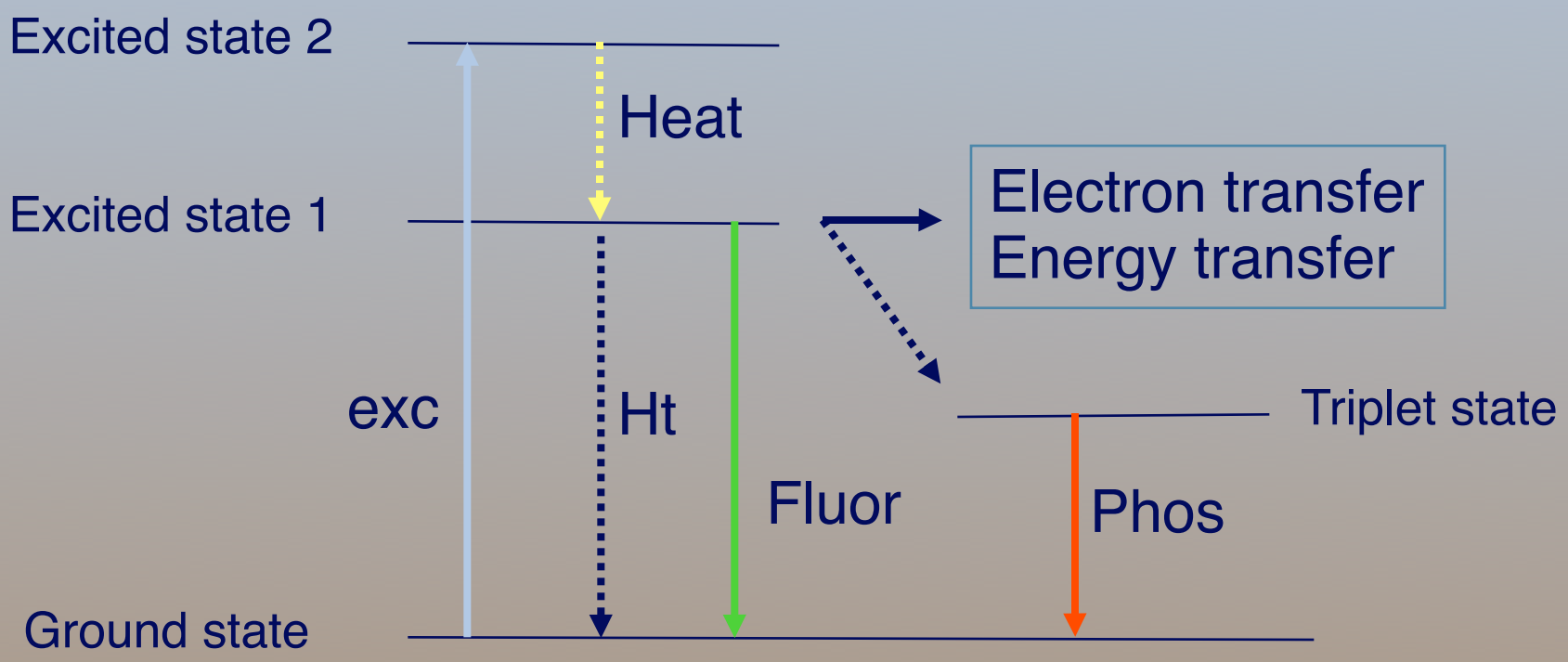


Figure 5. Pennsylvania sandstone with photosynthetic biofilm. Biofilm is less than 100 micrometers thick at greatest depth. Left, visible light. Center and right images are fluorescence images, emission at 820nm (near-infrared fluorescence) for both. Center, 365 nm excitation;30sec exposure; right, 665nm excitation, 3sec exposure. The 820 nm emission captures the near-infrared tail of the chlorophyll emission. Notice the much-improved contrast between background signal and chlorophyll fluorescence obtained with longer wavelength emission. This figure illustrates the sensitivity of detection possible even with weak excitation and shows that wavelength selection capability is essential for optimal contrast.

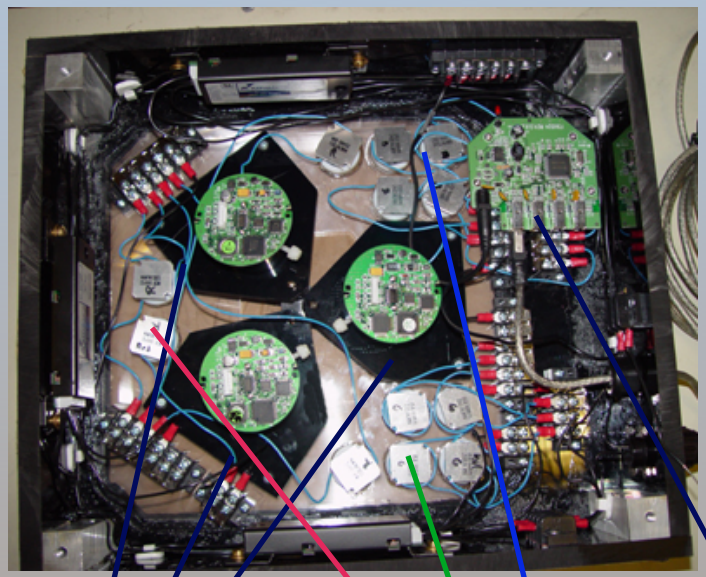
Excited states



Purpose of April Atacama field trip

- Search for life – get feel for challenges
- Find out what life exists at site
- Test imaging microscopes
  - Belly cam (on CMU rover….Wettergreen)
  - Research fluorescence imaging microscope
- Test reagents
- Learn what is required for next field trip

Rover bellycam – imaging hardware

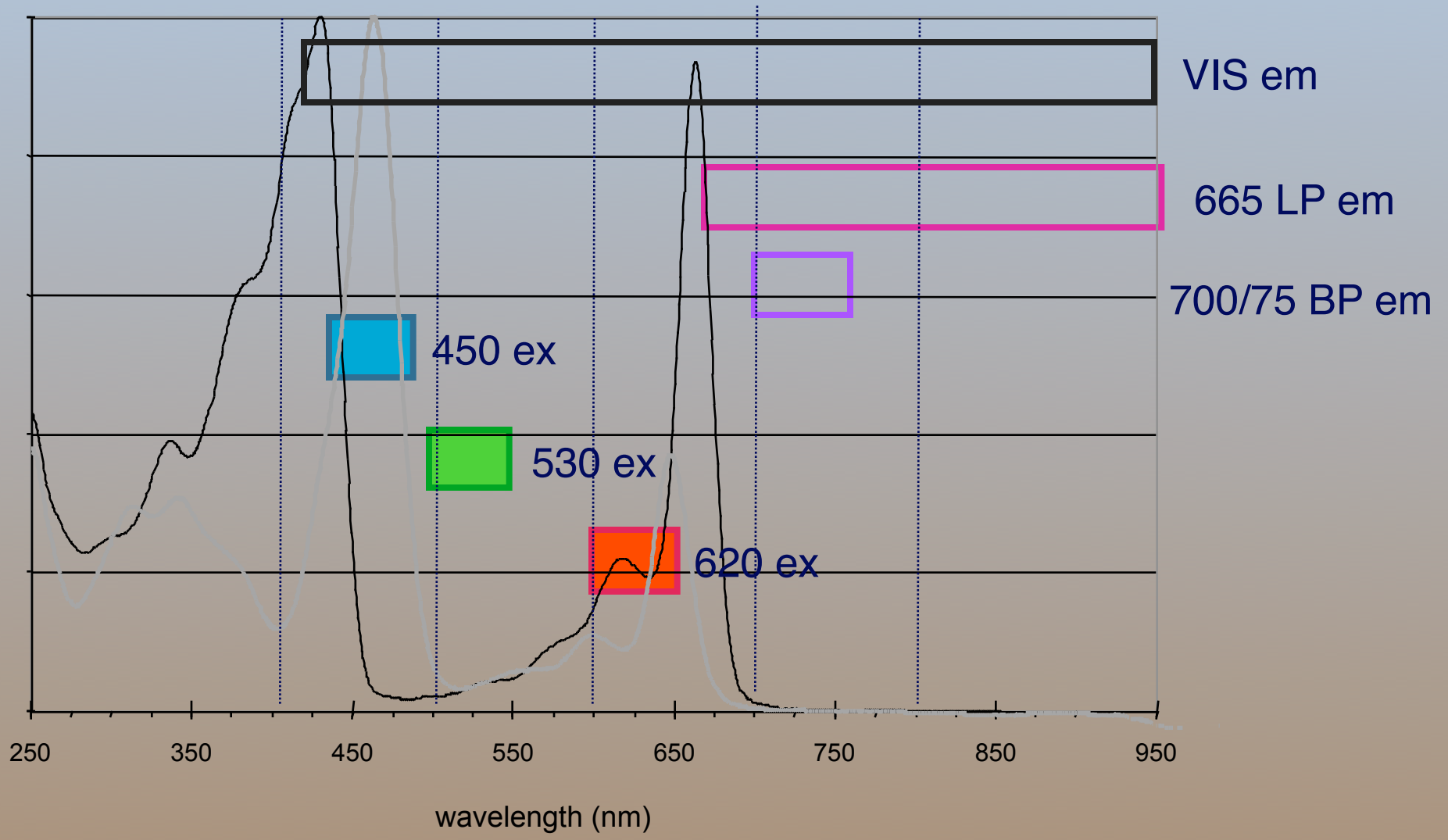


Web cams

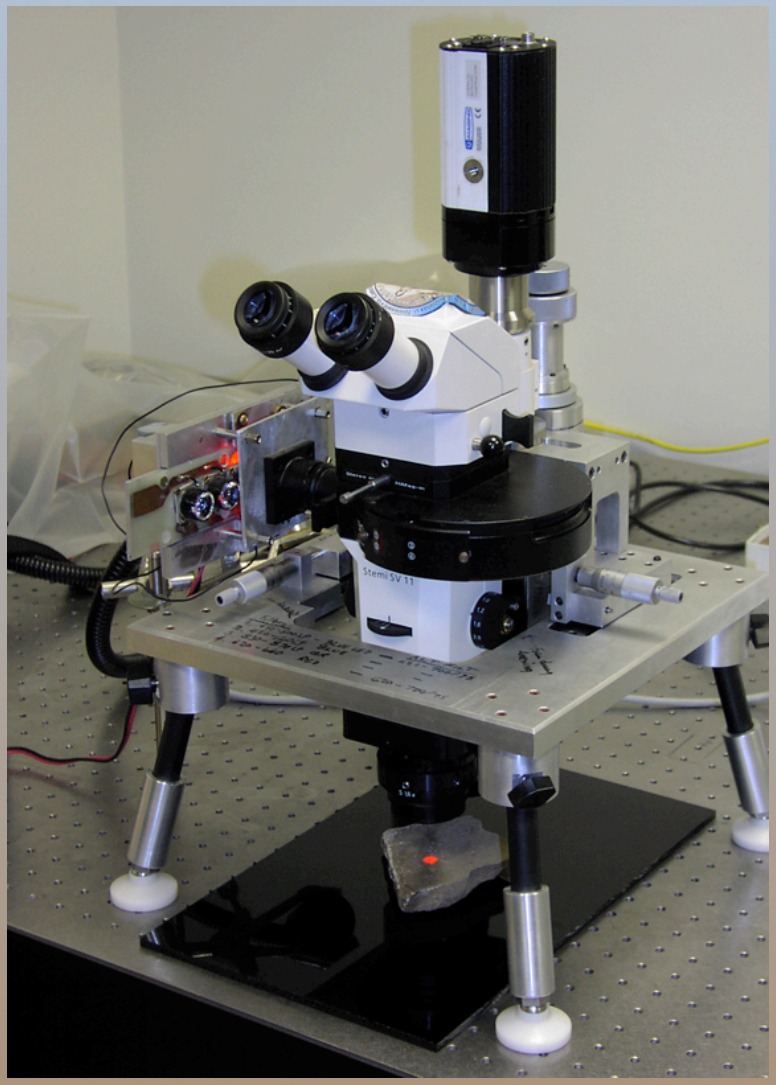
R G B
1W LEDs

Firewir
e
Hub

Optical filter sets - bellycam

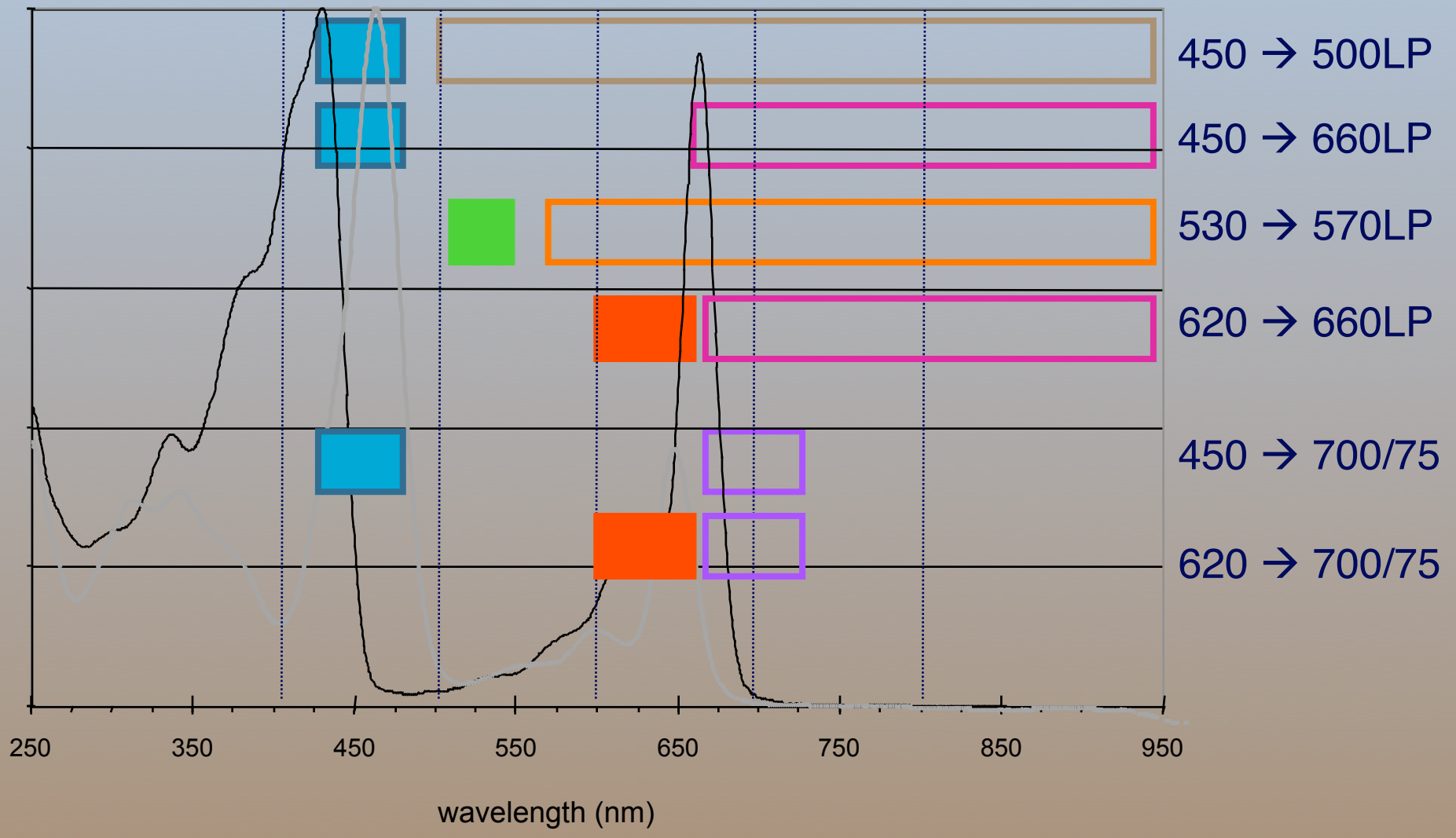


Picture of “ground truth”microscope



Yo Trey…What do you see?

Optical filter sets – ground truth microscope

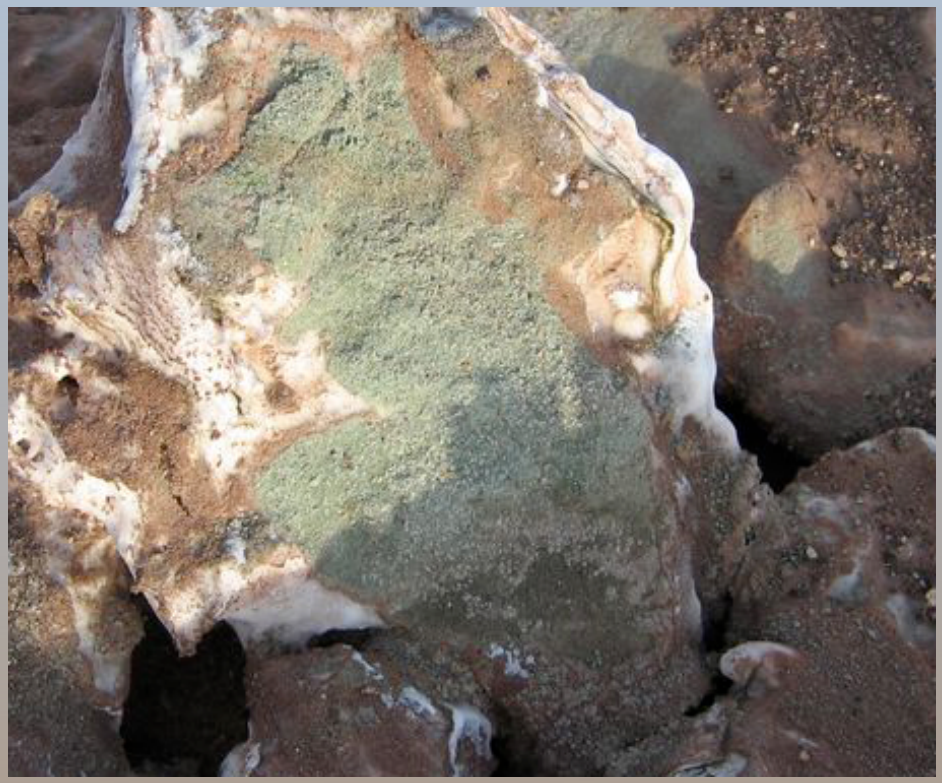


Yes…there was life.

Lichen!!
What the hell is a lichen??

Fog

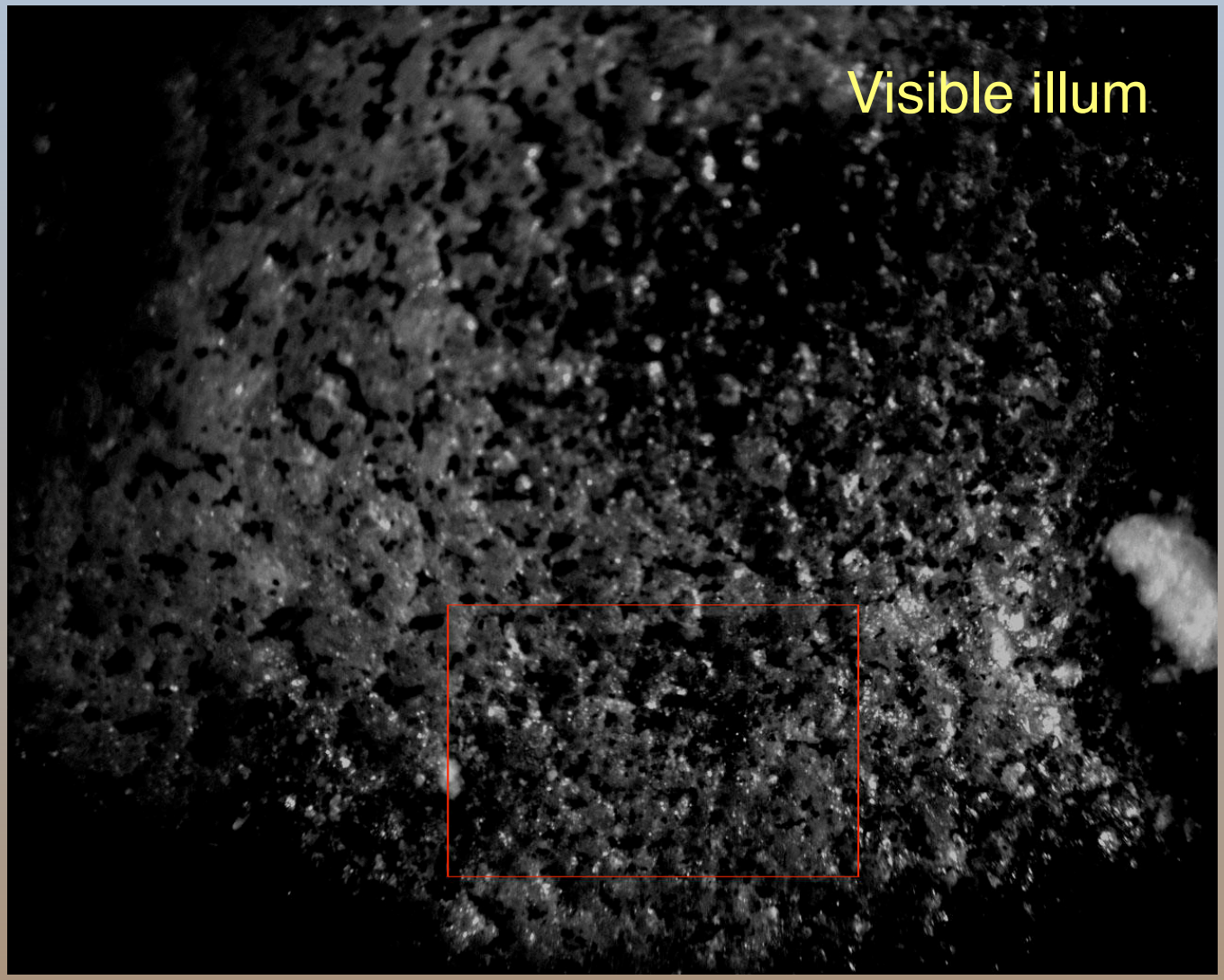
More examples



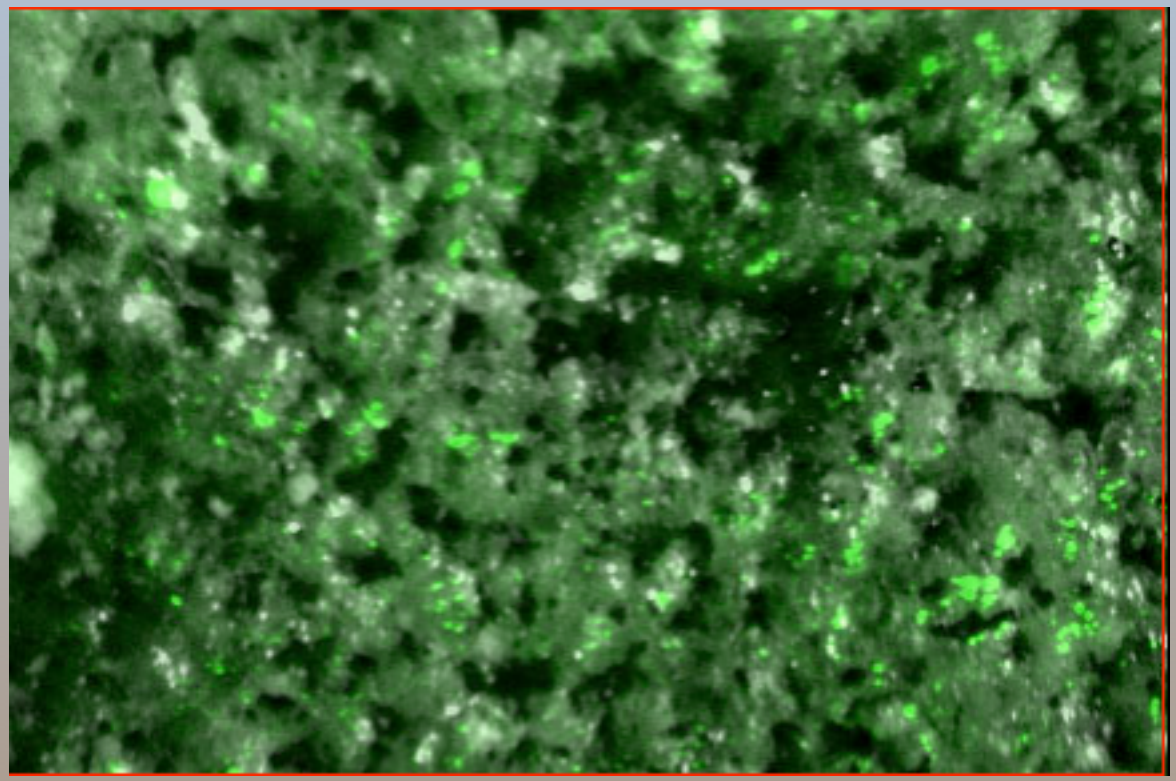
Lichens: Fun plus either algae or cyanobacteria

Images of samples

Sample 15



Sample 15



Vis + 450-500lp + 530-570lp + 620-660lp

Sample 2a

Sample 2a

Vis + 450-500lp

Vis + 620-660lp

Sample 2a-mag-red

Conclusions from sample images

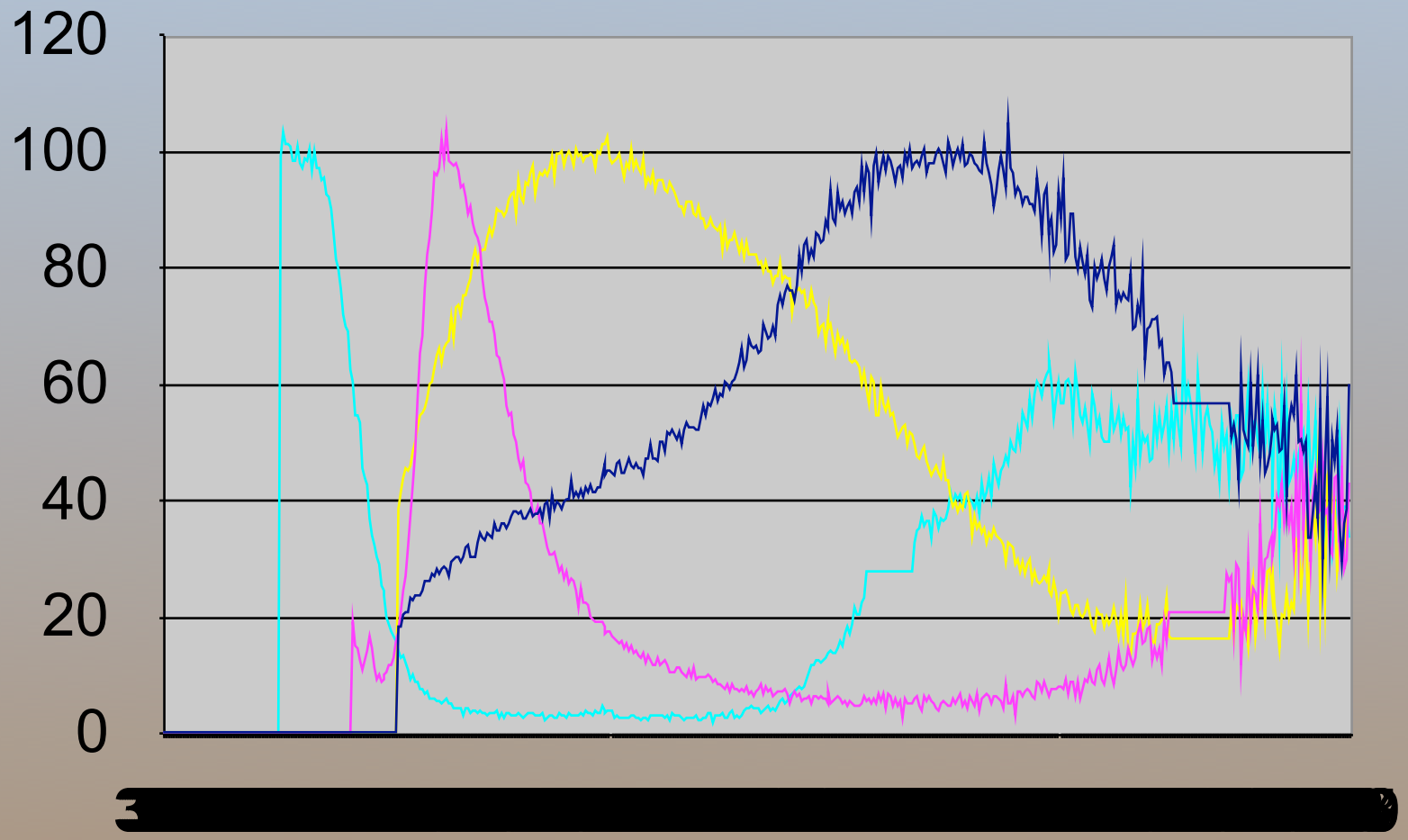
- **Some samples show almost no fluorescence**
  - **Nos. 5a, 11, 13, 14, 26**
- **Some samples fluorescence in all channels (incl. 600+nm)**
  - **Nos. 9, 10, 15, 27 (probable photosynthetic org.)**
- **Some samples show mainly green-orange-red fluoresc.**
  - **Nos. 5b, 6b, 2a, 8, 16 (possible phyco-cyanobacteria)**
- **Spritzing probes: too little experimentation**
- **Hence:**
  - **photosynthetic life probable in some samples**

What we learned from Atacama trip

- Improve ground truth imaging system
- Belly cam changes
  - Camera, filter improvements
  - Imaging system x, y, z movable on rover
  - Night and day operation
  - Spritzer system implementation
- Lichens key life form in one region
  - Fungi, cyanobacteria, algae…all photosynthetic will be found
- Need dye for polysaccharide coatings…spores etc
- Accessibility of target molecules to fluorescent probes
- Mineral fluorescence could be a problem

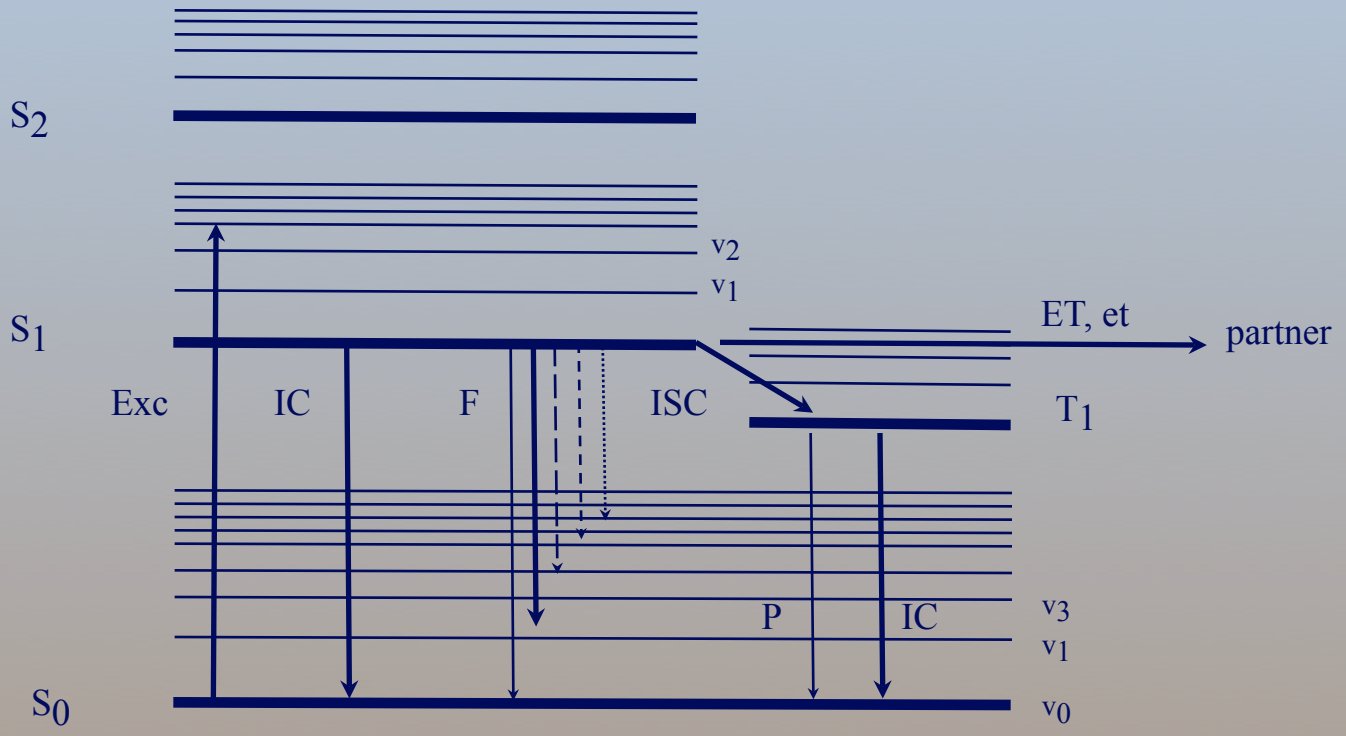
Four fluorescent mineral

Four fluorescent minerals



THE END

Energy level diagram



The horizontal lines in this diagram represent the energy content (levels) that a chromophore can have. Lighter horizontal lines are additional vibrational excitation the molecule can pick up. Upward arrows are photon absorption. “F” is fluorescence, “IC” is internal conversion, “ISC” is crossing to a “triplet state” where phosphorescence takes place, and “ET” is energy transfer to another chromophore.